ORIGINAL ARTICLE

Prevention of *M. tuberculosis* Infection with H4:IC31 Vaccine or BCG Revaccination

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ABSTRACT

BACKGROUND

Recent *Mycobacterium tuberculosis* infection confers a predisposition to the development of tuberculosis disease, the leading killer among global infectious diseases. H4:IC31, a candidate subunit vaccine, has shown protection against tuberculosis disease in preclinical models, and observational studies have indicated that primary bacille Calmette–Guérin (BCG) vaccination may offer partial protection against infection.

METHODS

In this phase 2 trial, we randomly assigned 990 adolescents in a high-risk setting who had undergone neonatal BCG vaccination to receive the H4:IC31 vaccine, BCG revaccination, or placebo. All the participants had negative results on testing for *M. tuberculosis* infection on the QuantiFERON-TB Gold In-tube assay (QFT) and for the human immunodeficiency virus. The primary outcomes were safety and acquisition of *M. tuberculosis* infection, as defined by initial conversion on QFT that was performed every 6 months during a 2-year period. Secondary outcomes were immunogenicity and sustained QFT conversion to a positive test without reversion to negative status at 3 months and 6 months after conversion. Estimates of vaccine efficacy are based on hazard ratios from Cox regression models and compare each vaccine with placebo.

RESULTS

Both the BCG and H4:IC31 vaccines were immunogenic. QFT conversion occurred in 44 of 308 participants (14.3%) in the H4:IC31 group and in 41 of 312 participants (13.1%) in the BCG group, as compared with 49 of 310 participants (15.8%) in the placebo group; the rate of sustained conversion was 8.1% in the H4:IC31 group and 6.7% in the BCG group, as compared with 11.6% in the placebo group. Neither the H4:IC31 vaccine nor the BCG vaccine prevented initial QFT conversion, with efficacy point estimates of 9.4% (P=0.63) and 20.1% (P=0.29), respectively. However, the BCG vaccine reduced the rate of sustained QFT conversion, with an efficacy of 45.4% (P=0.03); the efficacy of the H4:IC31 vaccine was 30.5% (P=0.16). There were no clinically significant between-group differences in the rates of serious adverse events, although mild-to-moderate injection-site reactions were more common with BCG revaccination.

CONCLUSIONS

In this trial, the rate of sustained QFT conversion, which may reflect sustained *M. tuberculosis* infection, was reduced by vaccination in a high-transmission setting. This finding may inform clinical development of new vaccine candidates. (Funded by Aeras and others; C-040-404 ClinicalTrials.gov number, NCT02075203.)

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†A list of investigators in the C-040-404 trial is provided in the Supplementary Appendix, available at NEJM.org.

Drs. Nemes, Geldenhuys, and Rozot and Drs. Scriba and Hatherill contributed equally to this article.

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Mycobacterium tuberculosis causes more deaths worldwide than any other infectious agent.[1] Vaccines that prevent pulmonary tuberculosis infection in young adults could have a major effect on the control of drug-sensitive and multidrug-resistant strains of the disease by interrupting transmission,[2] but the development of new vaccines has been hampered by the lack of validated preclinical models and human immune correlates of protection. *M. tuberculosis* exposure may result in either the early elimination of bacteria by innate or adaptive immunity or the establishment of infection, which may remain asymptomatic (latent) in most persons or progress to active disease.[3] Vaccine-mediated prevention of *M. tuberculosis* infection could be an important efficacy signal against tuberculosis disease.

The acquisition, persistence, and clearance of asymptomatic *M. tuberculosis* infection cannot be measured directly. The diagnosis of such infection is based on immunologic sensitization to *M. tuberculosis* antigens, as assessed by the tuberculin skin test and interferon-γ release assays. A test for *M. tuberculosis* infection, the QuantiFERON-TB Gold In-tube assay (QFT, Qiagen), suffers from assay variability and uncertainty regarding the most effective assay cutoff.[4,5] Recent infection, as diagnosed by means of the tuberculin skin test or QFT conversion, is associated with a higher risk of disease than is nonconversion or remote conversion (i.e., at least 2 years earlier).[5-8] Studies involving humans and animals have suggested that reversion to a negative tuberculin skin test is associated with early containment of *M. tuberculosis* infection and a lower risk of tuberculosis disease.[9-12] Although the clinical significance of QFT reversion remains to be established,[8] we propose that sustained QFT conversion more likely represents sustained *M. tuberculosis* infection and a higher risk of progression to disease than transient QFT conversion.

Observational studies have shown that primary bacille Calmette–Guérin (BCG) vaccination may offer partial protection against *M. tuberculosis* infection,[13-16] but this hypothesis has not been tested in randomized, placebo-controlled trials.[17] Two large, randomized trials showed no benefit of BCG revaccination for protection against tuberculosis disease,[18-20] but neither trial enrolled participants on the basis of *M. tuberculosis* infection status or measured infection acquisition during follow-up.

H4:IC31, a candidate subunit vaccine that consists of a recombinant fusion protein (H4) and IC31 adjuvant, signaling through toll-like receptor 9 (TLR9), contains mycobacterial antigens Ag85B and TB10.4, which do not cross-react with QFT. (Details regarding this vaccine are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.) H4:IC31 has shown protection in preclinical models[21-23] and acceptable safety and immunogenicity in humans.[24,25] In a phase 2, randomized, placebo-controlled clinical trial conducted in a high-risk setting for tuberculosis transmission, we evaluated the safety, immunogenicity, and prevention of initial and sustained QFT conversion by means of H4:IC31 vaccination or BCG revaccination in healthy South African adolescents without *M. tuberculosis* infection who had already received the neonatal BCG vaccine.[8]

METHODS

TRIAL DESIGN

From April 1, 2014, to May 25, 2015, at two sites in South Africa, we enrolled adolescents (between the ages of 12 and 17 years) who had received the BCG vaccine in infancy (Table 1). All the participants had negative results on QFT for *M. tuberculosis* infection and for the human immunodeficiency virus (HIV). Excluded were participants who had received previous treatment for tuberculosis or had current tuberculosis, who had a household contact with tuberculosis, who had substance abuse, or who were pregnant. All the participants provided written informed assent, and parents or legal guardians provided written informed consent. Regulatory approvals, consent procedures, and inclusion and exclusion criteria are described in the Supplementary Appendix.

Eligible participants were enrolled into two sequential cohorts, with each one randomly assigned in a 1:1:1 ratio to receive intramuscular H4:IC31 vaccine (15 μg H4 polyprotein [Sanofi Pasteur] and 500 nmol IC31 [Statens Serum Institut]) on day 0 and day 56, intradermal BCG vaccine (2×10^5 to 8×10^5 CFU [Statens Serum Institut]) on day 0, or intramuscular saline placebo on day 0 and day 56. In the first cohort of 90 participants (approximately 30 in each group), additional safety tests and immunogenicity assays were performed (see the Supplementary Appendix). The follow-up schedule for each participant was contingent on QFT results

Table 1. Characteristics of the Participants at Baseline (Safety Population).*

Characteristic	H4:IC31 Group (N=330)	BCG Group (N=330)	Placebo Group (N=329)	All Participants (N=989)
Site — no. (%)				
Emavundleni	24 (7.3)	25 (7.6)	23 (7.0)	72 (7.3)
SATVI	306 (92.7)	305 (92.4)	306 (93.0)	917 (92.7)
Median age (range) — yr	14 (12–17)	14 (12–17)	14 (12–17)	14 (12–17)
Race or ethnic group — no. (%)†				
Asian	1 (0.3)	1 (0.3)	1 (0.3)	3 (0.3)
Black	120 (36.4)	126 (38.2)	120 (36.5)	366 (37.0)
White	1 (0.3)	3 (0.9)	1 (0.3)	5 (0.5)
Cape mixed ancestry	208 (63.0)	200 (60.6)	207 (62.9)	615 (62.2)
Female sex — no. (%)	189 (57.3)	162 (49.1)	169 (51.4)	520 (52.6)
Median body-mass index (range)	19.6 (13.8–38.3)	19.4 (13.1–36.9)	19.9 (14.3–36.8)	19.6 (13.1–38.3)

* The participants' age, race, sex, and body-mass index (the weight in kilograms divided by the square of the height in meters) are reported for the two trial sites combined. SATVI denotes South African Tuberculosis Vaccine Initiative.
† Race or ethnic group was reported by the participants.

on day 84 and at months 6, 12, 18, and 24 (Fig. 1A). An 84-day washout period was stipulated to exclude participants who may have been infected with *M. tuberculosis* at baseline but who were not yet QFT-positive. Participants who tested QFT-positive on day 84 were followed for safety for 6 months after the last dose of vaccine but were excluded from efficacy evaluations. An independent data monitoring committee reviewed safety data obtained through day 7 and day 84 after vaccination from the first cohort and safety and efficacy data from all participants throughout the follow-up period. (Details are provided in the Supplementary Appendix.)

South African guidelines do not recommend the use of preventive antimicrobial agents in adults and children older than 5 years of agewho test positive for *M. tuberculosis* if they are HIV-negative. Thus, such therapy was not provided to participants who had QFT conversion.[26]

TRIAL OVERSIGHT

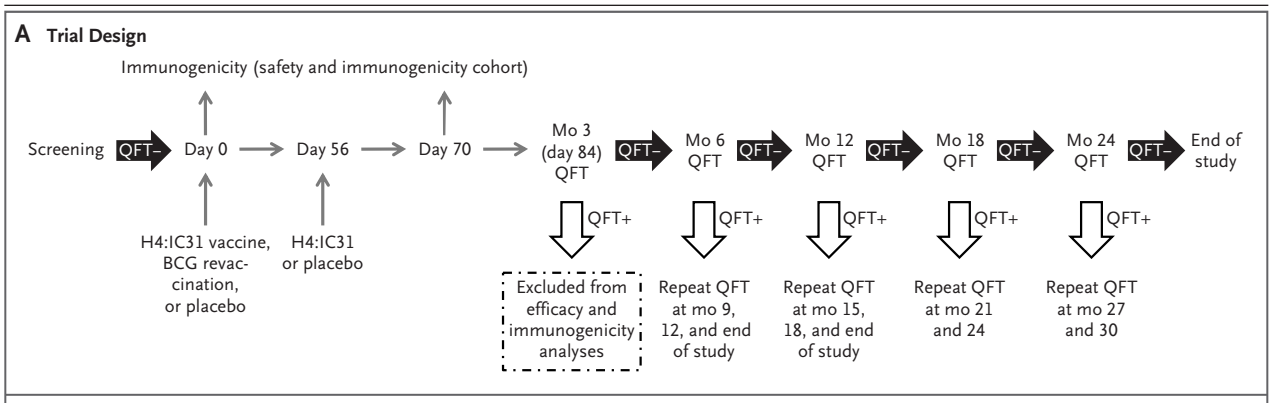
Aeras, a nonprofit biotechnology company focused on developing new tuberculosis vaccines, was the regulatory sponsor of the trial and contributed to the trial design and data analysis. The H4 antigen in the H4:IC31 vaccine was supplied by Sanofi Pasteur, and the IC31 adjuvant was supplied by Statens Serum Institut. The BCG vaccine (Statens Serum Institut) was purchased by each trial center. All the authors vouch for the accuracy and completeness of the data presented and for the fidelity of the trial to the protocol, which is available at NEJM.org.

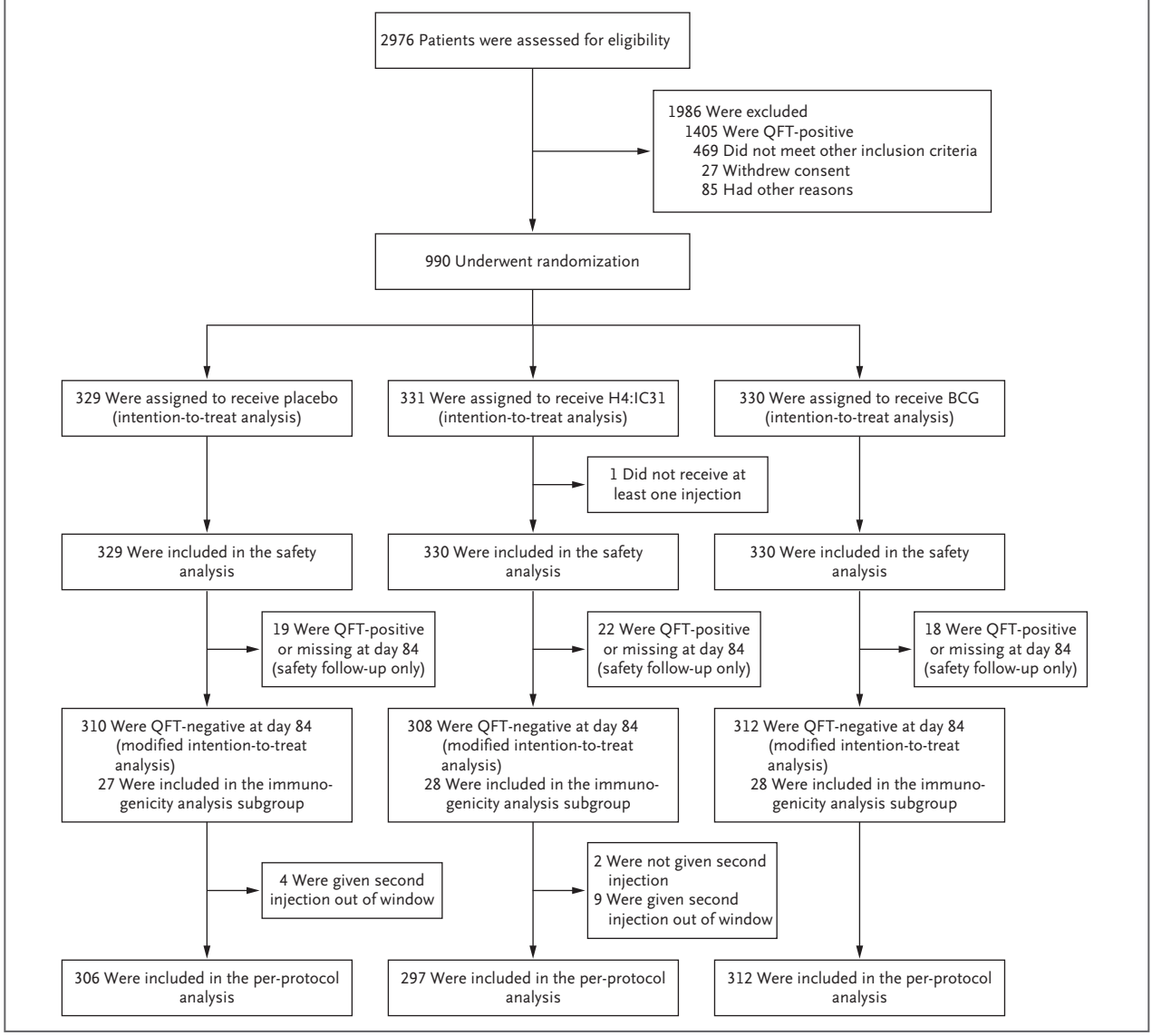
Figure 1 (facing page). Trial Design, Randomization, and Analyses.

Each participant followed a schedule of evaluations according to trial group (H4:IC31 vaccine, bacille Calmette–Guérin [BCG] revaccination, or placebo) and results on the QFT (QuantiFERON-TB Gold In-tube assay) for *Mycobacterium tuberculosis* infection, with testing performed on day 84 and months 6, 12, 18, and 24 (Panel A). A QFT conversion to a positive test was defined as a change from negative (<0.35 IU per milliliter) on day 84 to positive (≥0.35 IU per milliliter). The 84-day washout period was stipulated in order to exclude participants who might have been infected with *M. tuberculosis* at baseline but who were not yet QFT-positive. After the primary analysis, the independent data monitoring committee recommended that participants who had QFT conversion at month 6 or 12 should return for an additional end-of-trial visit to evaluate sustained QFT conversion. Safety outcomes were assessed at each trial visit, including the evaluation of symptoms of tuberculosis disease. Among the 2976 participants who had undergone screening, 1986 were excluded for one or more reasons (Panel B). The most common reason for ineligibility was a positive QFT test (in 1405 participants [71%]). Other common reasons for exclusion were abnormal blood results (in 244 participants [12%]), body-mass index out of the prespecified range (in 122 [6%]), and a previous diagnosis of tuberculosis or a household contact with tuberculosis (in 55 [3%]).

SAFETY OUTCOMES

We recorded solicited adverse events for 7 days, unsolicited adverse events for 28 days, and injection-site adverse events for 28 days after the administration of the H4:IC31 vaccine or placebo and for 84 days after BCG revaccination. Serious





adverse events and adverse events of special interest were recorded for the entire study period (see the Supplementary Appendix). A serious adverse event was defined as one that results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or substantial incapacity or disruption in the ability to conduct normal life functions, a congenital anomaly or birth defect, or an adverse event that jeopardizes the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. The severity of an adverse event was assessed on the basis of a toxicity table, as modified from a table published by the Division of AIDS of the National Institute of Allergy and Infectious Diseases for grading of the severity of adult and pediatric adverse events.

IMMUNOGENICITY OUTCOMES

Peripheral-blood mononuclear cells were stimulated with vaccine antigens (peptides spanning the sequence of Ag85B and TB10.4 or whole BCG vaccine), as well as negative and positive controls. This process was followed by intracellular cytokine staining with fluorescent antibodies[27] and data acquisition with the use of flow cytometry (Table S1 in the Supplementary Appendix).

EFFICACY OUTCOMES

We performed an efficacy assessment in the modified intention-to-treat population, which included all the participants who had received at least one dose of vaccine and who had not had QFT conversion by day 84. We considered that QFT conversion indicated the acquisition of *M. tuberculosis* infection and that sustained QFT conversion indicated sustained *M. tuberculosis* infection. The primary efficacy outcome was an initial QFT conversion, which was defined as an interferon-γ value of 0.35 IU or more per milliliter at any time after day 84 in the H4:IC31 group and BCG group, as compared with the placebo group. The QFT assay was performed according to the manufacturer's instructions, with the adoption of additional, more stringent limits to reduce variability and improve reproducibility.[5] (Details regarding this assay are provided in the Supplementary Appendix.)

The secondary efficacy outcome was sustained QFT conversion to a positive test without reversion to negative status at 6 months after the initial QFT conversion (i.e., three consecutive positive QFT results after day 84) (Fig. 1A). Exploratory efficacy outcomes included the evaluation of sustained conversion through the end of the trial and alternative QFT threshold values for initial QFT conversion, which were determined as follows: interferon-γ values of 0.20 IU per milliliter or more at any time after day 84, values of less than 0.2 IU per milliliter at any time before conversion and more than 0.7 IU per milliliter at any time after day 84, values of more than 0.7 IU per milliliter at any time after day 84, and values of more than 4.0 IU per milliliter at any time after day 84. The only alternative thresholds that were assessed for sustained QFT conversion were a value of less than 0.2 IU per milliliter at any time before conversion and a value of more than 0.7 IU per milliliter for three consecutive measures after day 84,[5,28] as detailed in the Supplementary Appendix.

RANDOMIZATION AND BLINDING

Trial-group assignments were concealed by an interactive Web-response system. The assignment was based on block randomization in a 1:1:1 ratio to the H4:IC31 group, BCG group, or placebo group, stratified according to school (South African Tuberculosis Vaccine Initiative at the Worcester site) or residential area (Emavundleni site). Blinding was partial because BCG causes a recognizable injection-site reaction and is administered once. However, randomization to receive the H4:IC31 vaccine or placebo was double-blind: syringe contents were masked, injection volumes were identical, and injections were administered by a research nurse who did not perform trial procedures or data collection after enrollment. Laboratory personnel were unaware of all trial-group assignments.

STATISTICAL ANALYSIS

We determined the sample size on the basis of the reduction in the rate of *M. tuberculosis* infection, as defined by the initial QFT conversion. The trial was designed to provide a power of 80% to distinguish a 50% lower rate of QFT conversion in the H4:IC31 group or in the BCG group than in the placebo group. We used a one-sided type I error rate of 0.10 to minimize the risk of a false negative signal at the expense of a false positive signal, thus prioritizing the detection of a proof-of-concept efficacy signal for decision making regarding further clinical de-

velopment of either vaccine.[9] Therefore, we report two-sided confidence intervals of both 95% and 80%. The trial was not powered to distinguish a difference in efficacy between the H4:IC31 group and the BCG group. We determined that a sample size of 330 participants per group would provide 64 initial QFT conversion outcomes approximately 21 months after the enrollment of the first participant.

We used two log-rank statistics (for the H4:IC31 group and the BCG group versus the placebo group) to analyze the primary and secondary efficacy outcomes without adjustment for multiple comparisons (see the Supplementary Appendix). Vaccine efficacy estimates are based on hazard ratios that were calculated from a Cox regression model (i.e., vaccine efficacy equals 1 minus the hazard ratio). All the analyses presented here have been evaluated at a two-sided alpha level of 0.05. Because the trial was powered at a one-sided alpha level of 0.10, we also present one-sided P values for the primary and secondary efficacy outcomes. Details regarding all the analyses and outcomes are provided in the Supplementary Appendix. Data management and statistical analyses were performed by a contract research organization (IQVIA) and the trial statistician.

RESULTS

TRIAL PARTICIPANTS

Of the 2976 participants who underwent screening, 990 were enrolled. Among the 1986 volunteers who were excluded from participation, 1405 (71%) had positive QFT results (Fig. 1B). There were no significant differences among the groups at baseline (Table 1). The final visit occurred on August 28, 2017. A total of 41 participants (4%) were lost to follow-up during the trial.

SAFETY

Safety was assessed in all participants who had received at least one dose of a trial vaccine or placebo. A total of 550 participants had at least one adverse event (Tables S2 and S3 in the Supplementary Appendix). The types of adverse events were similar in the H4:IC31 group and the placebo group. Adverse events were more frequent in the BCG group, with 98.8% having at least one event. These events were predominantly local injection-site reactions of mild-to-moderate severity, a finding that was consistent with the known reactogenicity profile of the BCG vaccine.[29] The rate of upper respiratory tract infections was lower in the BCG group than in either the H4:IC31 group or the placebo group (2.1%, 9.4%, and 7.9%, respectively; P<0.001 for both comparisons). In total, there were 4 severe adverse events (1 each in the H4:IC31 group and the BCG group and 2 in the placebo group) and 19 serious adverse events, none of which were deemed by investigators to be related to a trial vacccine. No adverse events of special interest were reported during the trial. There was no clinically significant difference in the rate of severe adverse events or serious adverse events among the three trial groups. There was one death from suicide of a participant in the placebo group.

IMMUNOGENICITY

Frequencies of cytokine-expressing antigen-specific T cells were assessed at baseline and on day 70 by means of intracellular cytokine staining (Fig. 2). In the H4:IC31 group, CD4+ T-cell responses that were specific for mycobacterial antigens Ag85B and TB10.4 were low before vaccination, and the administration of H4:IC31 induced significant increases in these responses. By contrast, in the BCG group, high levels of prevaccination BCG-specific CD4+ T-cell responses were observed in all three groups, and BCG revaccination significantly boosted the BCG-specific CD4+ T-cell responses (Fig. 2, and Fig. S1 in the Supplementary Appendix).

EFFICACY

In the three trial groups, 930 participants were included in the modified intention-to-treat population after the exclusion of 59 participants who had positive results on QFT or missing data on day 84 and 1 participant who did not receive at least one vaccine dose (Fig. 1B). There were 134 initial QFT conversions (14.4%), for a rate of 9.9 per 100 person-years (Fig. S2A in the Supplementary Appendix), with a high QFT reversion rate (in 48 of 133 participants [36.1%] who underwent repeated QFT). A total of 82 participants had sustained QFT conversion (8.8% of all participants; 62.6% of those with an initial conversion for whom QFT results were available) (Fig. 3A). Among the participants with an initial QFT conversion, the median time until such conversion was 15.0 months. No cases of tuberculosis disease were identified.

Neither H4:IC31 vaccination nor BCG revac-

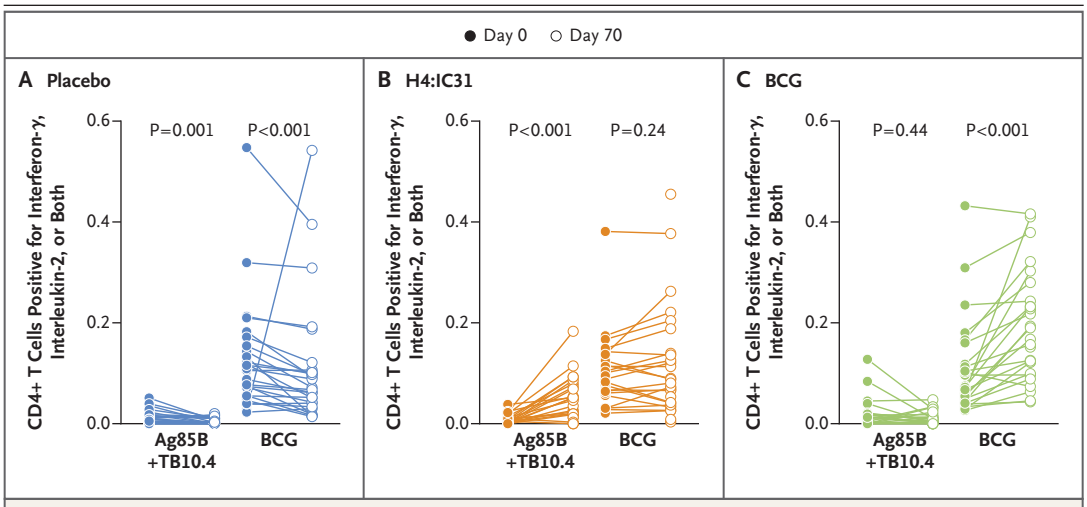


Figure 2. Immunogenicity.

Shown are immunogenicity levels in the three trial groups, as measured on intracellular cytokine staining and flow cytometry in peripheral-blood mononuclear cells after stimulation with Ag85B or TB10.4 peptide pools or BCG. Paired responses of CD4+ T cells expressing interferon-γ, interleukin-2, or both for each participant are shown on day 0 (solid circles) and day 70 (open circles) in the H4:IC31 group, the BCG group, and the placebo group, with 23 to 28 participants included in each group at each time point. Changes in response that occurred between day 0 and day 70 were compared with the use of the Wilcoxon signed-rank test.

cination met the primary efficacy criterion on the basis of initial QFT conversion rates (Table 2 and Fig. 3B). In the H4:IC31 group, the vaccine efficacy point estimate for the prevention of sustained QFT conversion (a secondary outcome) was 30.5% (95% confidence interval [CI], –15.8 to 58.3) and did not differ significantly from that of placebo (P=0.16) (Table 2 and Fig. 3C); among the participants with QFT conversion, reversions occurred in 17 of 43 participants (39.5%) for whom data were available. The efficacy of the H4:IC31 vaccine for the prevention of sustained QFT conversion at the end of the trial was 34.2% (95% CI, –10.4 to 60.7; P=0.11) (Table 2).

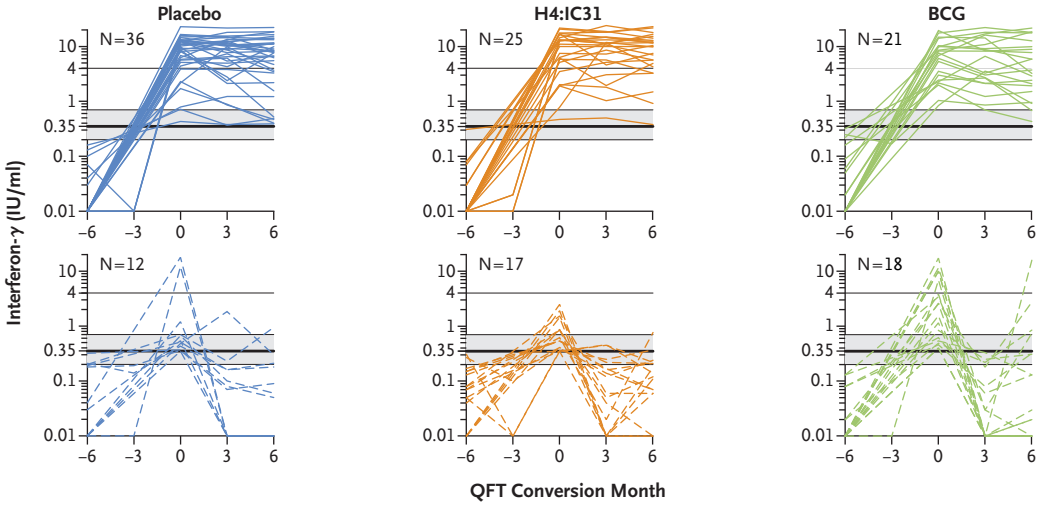
In the BCG group, the efficacy of revaccination for the prevention of sustained QFT conversion was 45.4% (95% CI, 6.4 to 68.1; P=0.03) (Table 2 and Fig. 3C); 48.2% efficacy was observed at the end of the trial (95% CI, 10.5 to 70.0; P=0.02) (Table 2). This BCG-induced effect was explained by a 6-month QFT reversion rate after conversion that was nearly twice as high as that in the placebo group (19 of 41 participants [46.3%] vs. 12 of 49 participants [24.5%]). Among all the reversions, 88% had occurred by 3 months after conversion (Fig. 3D).

In exploratory analyses, the vaccine efficacy for a sustained QFT conversion on the basis of a stringent QFT conversion threshold (<0.2 IU per

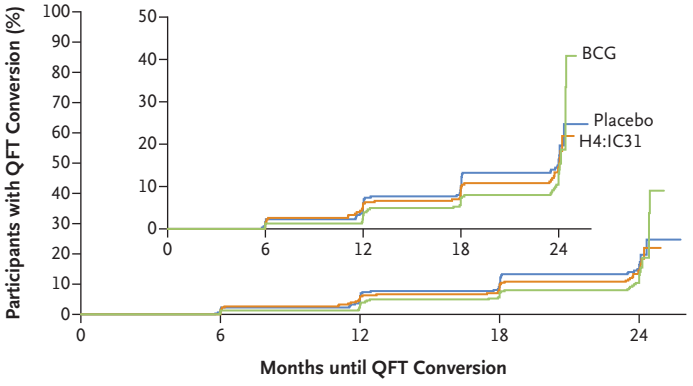
Figure 3 (facing page). Vaccine Efficacy.

Panel A shows longitudinal quantitative interferon-γ values, as measured on QFT, in each trial group, according to the time point of the initial QFT conversion (month 0). Each line represents data for one participant; not shown are data for participants who did not have a QFT conversion and those who had missing QFT results after an initial conversion. The solid lines (top row) indicate participants who met the secondary efficacy outcome (sustained QFT conversion), and the dashed lines (bottom row) indicate participants who had an initial QFT conversion and then reverted to a negative test. The solid black horizontal line denotes the manufacturer's recommended threshold for test positivity (0.35 IU per milliliter), with the shaded areas indicating the range of QFT values (0.2 to 0.7) in which the test result is considered to be uncertain.[5] The gray horizontal line at 4.0 IU per milliliter denotes an alternative QFT threshold that was applied in exploratory analyses. Values of less than 0.01 IU per milliliter were included with the 0.01 measure to enable plotting on the log scale. Panel B shows Kaplan–Meier curves representing the time until initial QFT conversion (primary efficacy outcome) after the first dose of vaccine, according to trial group in the modified intention-to-treat population. The inset shows the same data on an expanded y axis. Panel C shows Kaplan–Meier curves representing the time until an initial QFT conversion in participants who had a sustained conversion (secondary efficacy outcome), according to trial group in the modified intention-to-treat population. Panel D shows the time until QFT reversion within 6 months after an initial conversion in participants who had available QFT values at 3 months and 6 months after conversion.

A Interferon-γ Values, According to Timing of QFT Conversion

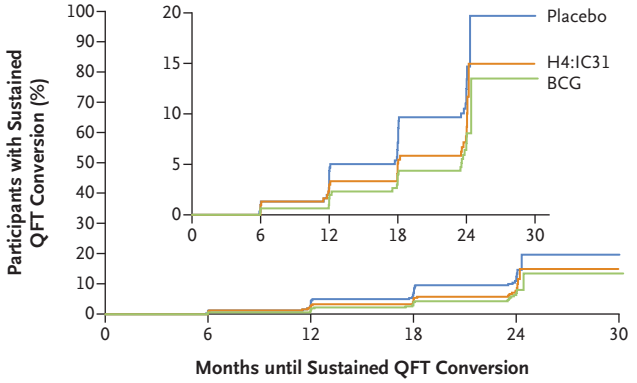


B Initial QFT Conversion



No. at Risk					
Placebo	310	301	283	261	120
H4:IC31	308	303	281	265	120
BCG	312	308	294	276	134

C Sustained QFT Conversion



No. at Risk					
Placebo	310	302	287	263	122
H4:IC31	308	303	288	268	124
BCG	312	308	297	281	136

D No QFT Reversion to Negative Test within 6 Months after Initial Conversion

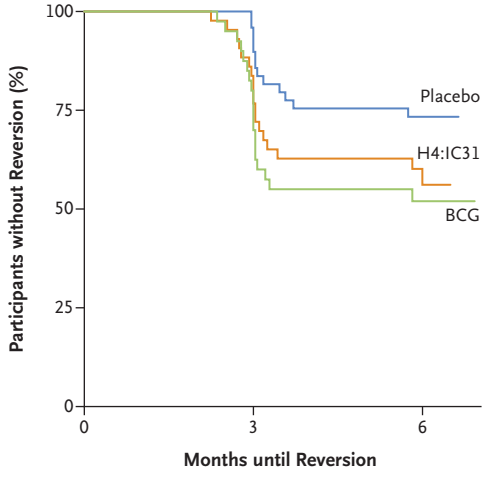


Table 2. Vaccine Efficacy.*

Outcome	QFT Conversion Threshold	H4:IC31 Group				BCG Group				Placebo Group
			Vaccine Efficacy				Vaccine Efficacy			
	IU/ml	*no./total no. (%)*	*point estimate (%)*	*80% CI (one-sided P value)†*	*95% CI (two-sided P value)‡*	*no./total no. (%)*	*point estimate (%)*	*80% CI (one-sided P value)†*	*95% CI (two-sided P value)‡*	*no./total no. (%)*
QFT conversion: primary outcome§	≥0.35	44/308 (14.3)	9.4¶	−18.3 to 30.6 (0.32)	−36.2 to 39.7 (0.63)	41/312 (13.1)	20.1¶	−4.8 to 39.1 (0.14)	−21.0 to 47.2 (0.29)	49/310 (15.8)
Sustained QFT conversion: secondary outcome‖	≥0.35	25/308 (8.1)	30.5¶	3.0 to 50.2 (0.08)	−15.8 to 58.3 (0.16)	21/312 (6.7)	45.4¶	22.3 to 61.6 (0.01)	6.4 to 68.1 (0.03)	36/310 (11.6)
Exploratory outcome										
Sustained QFT conversion**	<0.2 to >0.7	24/308 (7.8)	23.2¶	−8.8 to 45.8 (0.16)	−30.9 to 54.9 (0.33)	19/312 (6.1)	41.6¶	15.2 to 59.8 (0.03)	−3.3 to 67.0 (0.06)	31/310 (10.0)
End-of-trial sustained QFT conversion††	≥0.35	24/308 (7.8)	34.2¶	7.7 to 53.0 (0.05)	−10.4 to 60.7 (0.11)	20/312 (6.4)	48.2¶	25.9 to 63.8 (0.008)	10.5 to 70.0 (0.02)	36/310 (11.6)
QFT conversion‡‡	>4.0	22/308 (7.1)	34.5§§	6.8 to 54.2 (NA)	−12.1 to 62.3 (0.13)¶¶	19/312 (6.1)	45.1§§	20.5 to 62.2 (NA)	3.8 to 69.3 (0.04)¶¶	33/310 (10.6)
QFT conversion in ITT population‖‖	≥0.35	63/331 (19.0)	6.0¶	−17.7 to 24.9 (0.36)	−32.6 to 33.4 (0.72)	57/330 (17.3)	16.7¶	−4.9 to 33.9 (0.15)	−18.5 to 41.5 (0.30)	67/329 (20.4)

* All analyses were performed in the modified intention-to-treat (ITT) population unless otherwise indicated. A QFT (QuantiFERON-TB Gold In-tube assay) conversion is defined as a change from negative (<0.35 IU per milliliter) on day 84 to positive (≥0.35 IU per milliliter), unless the threshold for conversion is otherwise indicated. NA denotes not applicable according to the statistical analysis plan.

† P values were calculated with the use of a one-sided log-rank test, as compared with placebo, without adjustment for multiple comparisons.

‡ P values were calculated with the use of a two-sided log-rank test, as compared with placebo, without adjustment for multiple comparisons.

§ Data are for participants who had a QFT conversion at any time after day 84.

¶ The vaccine efficacy point estimate and 80% and 95% confidence intervals are based on the hazard ratio estimated from the Cox regression model.

‖ Data are for participants who had a sustained QFT conversion without reversion by 6 months after the initial QFT conversion (i.e., three consecutive positive QFT results after day 84), with the exclusion of data collected during the end-of-trial callback visit for participants who had a conversion at 6 months or 12 months.

** Data are for participants who had a QFT conversion at any time during the trial on the basis of an alternative threshold of less than 0.2 IU per milliliter at any time point before conversion and more than 0.7 IU per milliliter at the time of conversion and maintained the QFT value of more than 0.7 IU per milliliter through 6 months after the initial conversion, with the exclusion of data collected during the end-of-trial callback visit for participants who had a conversion at 6 months or 12 months.

†† Data are for participants who had a QFT conversion at any time point during the trial and did not have a change in the QFT value from positive to negative through the end of the study, with the inclusion of data collected during the end-of-study callback visit for participants who had a conversion at 6 months or 12 months.

‡‡ Data are for participants who had a QFT conversion to more than 4.0 IU per milliliter at any time after day 84.

§§ Vaccine efficacy point estimates and 95% confidence intervals were calculated with the use of the conditional binomial procedure.

¶¶ Two-sided P values were calculated with the use of the Pearson chi-square test.

‖‖ Data are for participants in the ITT population who had a QFT conversion at any point during the trial.

milliliter to >0.7 IU per milliliter) was 23.2% in the H4:IC31 group (95% CI, −30.9 to 54.9; P=0.33) and 41.6% in the BCG group (95% CI, −3.3 to 67.0; P=0.06). The vaccine efficacy for conversion on the basis of the most stringent QFT threshold (>4.0 IU per milliliter) was 34.5% (95% CI, −12.1 to 62.3; P=0.13) in the H4:IC31 group and 45.1% (95% CI, 3.8 to 69.3; P=0.04) in the BCG group (Table 2, and Fig. S2B in the Supplementary Appendix). The results of additional exploratory analyses are provided in Table S4 and Figure S2C in the Supplementary Appendix. In unplanned post hoc analyses, estimates of efficacy that were based on primary and secondary outcomes were not affected by sex, race, or trial site.

DISCUSSION

In this randomized, controlled trial of two vaccines to prevent *M. tuberculosis* infection, we found that vaccination with either agent reduced the rate of sustained QFT conversion in a high-transmission setting, although neither the H4:IC31 vaccine nor BCG revaccination prevented initial QFT conversion. The efficacy estimate for the H4:IC31 vaccine (30.5%) against sustained QFT conversion did not meet standard statistical criteria for efficacy. However, our data indicate that at the prespecified 80% confidence level, the efficacy estimate for the H4:IC31 vaccine ranged from 3.0 to 50.2%. This observation suggests that subunit vaccines that include few antigens against *M. tuberculosis* may have a biologic effect, a finding that encourages clinical evaluation of next-generation subunit vaccine candidates.

BCG revaccination had 45.4% efficacy against sustained QFT conversion. The durability of this important finding and potential public health significance for protection against tuberculosis disease warrants epidemiologic modeling and further clinical evaluation. We found that vaccine-mediated protection against sustained QFT conversion may inform the clinical development of vaccine candidates before entry into larger-scale efficacy trials for disease prevention. Our findings, and the availability of stored biospecimens, also provide a potential opportunity to discover immune responses that correlate with protection against sustained QFT conversion, which could enable new methods for the design and evaluation of tuberculosis vaccines.

The efficacy signal for BCG revaccination was also observed for protection against conversion at an interferon-γ level of more than 4.0 IU per milliliter. This threshold was associated with an increased risk of tuberculosis disease in infants and adults,[28,30] a finding that was consistent with predictions from studies in animal models.[31]

A meta-analysis of observational studies of primary BCG vaccination showed a pooled estimate of 27% efficacy against initial *M. tuberculosis* infection and 71% efficacy against tuberculosis disease.[16] The efficacy of the primary BCG vaccine against disease is highly variable in different populations; efficacy is thought to be greatest in persons without previous mycobacteria exposure[32] and may last for 10 years.[32,33] Our findings suggest that BCG revaccination of QFT-negative adolescents may provide additional benefit.[17] Two large, cluster-randomized trials that evaluated the prevention of disease by BCG revaccination did not show efficacy.[19,20] However, neither trial enrolled participants on the basis of the status of either *M. tuberculosis* or HIV infection or tested for previous mycobacterial sensitization or acquisition of *M. tuberculosis* infection. In Brazilian children between the ages of 7 and 14 years, the efficacy of BCG revaccination against tuberculosis disease was 9% after 5 years[19] and 12% after 9 years, and neither estimate was significant.[18] The trial was cluster-randomized and open-label with no placebo group, and the outcome of the development of tuberculosis disease was determined from health-service records.[19] However, a modestly significant efficacy signal (33%) was observed in children who were revaccinated before the age of 11 years at one of two sites.[18] The second trial, a double-blind, randomized, placebo-controlled trial of BCG revaccination involving more than 46,000 participants between the ages of 3 months and 70 years showed no significant efficacy against confirmed tuberculosis disease (incidence rate ratio, 1.43)[20] in a Malawian community in which a trial of primary BCG vaccination had also shown no efficacy.[34]

On the basis of our results and given the substantial differences in trial methods, tuberculosis epidemiology, and study populations, a trial of BCG revaccination for the prevention of disease in adolescents who do not have *M. tuberculosis* infection is justified in settings with a high incidence of tuberculosis. Such a trial would also validate the strategy of evaluating the prevention of *M. tuberculosis* infection to increase the chances

of success of subsequent trials for the prevention of tuberculosis disease and to allow for possible identification of immune correlates of protection against disease. From a public health perspective, the potential risk of BCG disease among adolescents at high risk for HIV infection should be balanced against the potential benefits of BCG vaccination.

A successful tuberculosis vaccine might function by means of several mechanisms, including the prevention of initial *M. tuberculosis* infection, sustained infection, or progression to disease. Our results indicate that vaccination did not avert initial acquisition of infection by innate immune mechanisms but allowed the trafficking of antigens to lymphoid tissues to trigger adaptive immunity (as measured by an initial QFT conversion). Rather, we hypothesize that vaccine-mediated QFT reversion to negative status was associated with enhanced bacterial control or clearance, which was probably mediated by collaborative adaptive and innate immune responses (as have been associated with complete clearance of bacteria from individual granulomas in nonhuman primates).[35,36] Although antigen-specific memory T cells that are measured on QFT can persist after bacterial clearance,[31] there is a positive correlation between the replication of *M. tuberculosis* in animal models and the magnitude of interferon-γ responses to antigens that are specific to *M. tuberculosis*.[23] Indeed, in both humans and guinea pigs, transient conversion on the tuberculin skin test has been associated with a lower risk of tuberculosis disease than sustained conversion.[10-12] Further studies are required to understand the clinical significance of QFT reversion and the underlying immunologic determinants. Comprehensive analyses are required to elucidate immune responses and mechanisms that correlate with protection in order to guide the evaluation and design of new tuberculosis vaccines.

A definitive interpretation of our findings is limited because there is no definitive test for acquisition, persistence, or clearance of *M. tuberculosis* infection. QFT has technical limitations, which we addressed by implementing stringent assay procedures[5] and by using alternative threshold definitions and serial testing. Testing only for initial QFT conversion in this trial would not have shown efficacy; thus, in future trials that test vaccine efficacy for the prevention of *M. tuberculosis* infection, investigators may consider an evaluation of the prevention of sustained QFT conversion.

A trial that is designed to evaluate the prevention of *M. tuberculosis* infection has the potential to miss the effects of a vaccine that prevents tuberculosis disease but not *M. tuberculosis* infection.[16] Conversely, a vaccine that prevents infection mainly in the approximately 90% of persons with *M. tuberculosis* infection in whom disease never develops would have little effect on tuberculosis prevention.[9,37]

These findings support model predictions that vaccine efficacy against *M. tuberculosis* infection can be observed in a setting with very high transmission of the disease.[9] It is unclear whether our observations are generalizable to settings with a lower rate of transmission.[19,20]

Our results raise important questions with respect to the prevention of *M. tuberculosis* infection for the control of tuberculosis disease and provide a promising signal for BCG vaccine. These encouraging findings provide an impetus to reevaluate the use of BCG revaccination of populations that are free of *M. tuberculosis* infection for the prevention of disease.[17] The results may also inform the development of new tuberculosis vaccines and illustrate the value of conducting human trials of tuberculosis vaccine candidates.

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APPENDIX

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